The effect of body condition on serum concentrations of two teratogenic alkaloids (anagyrine and ammodendrine) from lupines (Lupinus species) that cause crooked calf disease

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J Anim Sci 2008.86:2771-2778.
doi: 10.2527/jas.2007-0610 originally published online Jun 6, 2008;

The effect of body condition on serum concentrations of two teratogenic alkaloids (anagyrine and ammodendrine) from lupines (*Lupinus* species) that cause crooked calf disease[1]

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ABSTRACT: Several species of lupine (*Lupinus* spp.) are toxic to livestock, causing death losses in sheep and cattle but more commonly crooked calf disease in pregnant range cows. The major toxic alkaloids in lupine are of the quinolizidine alkaloid group and include the teratogen anagyrine, which is primarily responsible for crooked calf disease. Lupines also contain teratogenic piperidine alkaloids including ammodendrine. Previous work in sheep has shown that lupine alkaloid clearance may be influenced by the animal's physiological status. Therefore, the purpose of this study was to determine if differences in body condition of cattle would alter the absorption and elimination of anagyrine or ammodendrine given in a single oral dose as *Lupinus leucophyllus* or *Lupinus sulphureus*, respectively. Mature nonlactating cows in low body condition (LBC, n = 4) and high body condition (HBC, n = 4) received a single dose of dry ground lupine plant (2.0 g/kg of BW) via oral gavage. *Lupinus leucophyllus* (anagyrine) was dosed first; then after 21 d the same animals were dosed with *L. sulphureus* (ammodendrine). Blood samples were taken via jugular venipuncture 0 to 60 h after dosing. Serum anagyrine and ammodendrine concentrations were evaluated. The concentration of anagyrine was greater ($P = 0.001$) in the HBC group and peaked 2 h after dosing versus 12 h in LBC cows. Similarly for ammodendrine, the alkaloid concentration peaked at 3 h after dosing for the HBC group compared with 6 h for the LBC group ($P = 0.001$). Area under the curve tended to differ ($P \leq 0.11$) for both alkaloids in the HBC group compared with the LBC group. There were also differences in the maximum serum anagyrine ($P = 0.02$) and ammodendrine ($P = 0.06$) concentrations. Elimination half-life (E1/2) tended to differ ($P = 0.12$) between the HBC and LBC groups for ammodendrine. The kinetic profiles suggest that body condition influenced the disposition of these alkaloids. This study also suggests that body condition may impact the risk of toxicity, teratogenicity, or both of these alkaloids.

Key words: alkaloid, ammodendrine, anagyrine, body condition, cattle, lupine

J. Anim. Sci. 2008. 86:2771–2778
doi:10.2527/jas.2007-0610

INTRODUCTION

Consumption of toxic plants by livestock can have a severe economic impact on livestock producers through death losses, abortions, and birth defects. Lupines (*Lupinus* spp.) that contain either of the teratogenic alkaloids, anagyrine or ammodendrine, can cause crooked calf disease when ingested by pregnant cows during gestation d 40 to 100 (Keeler, 1976; Keeler and Panter, 1989; Panter et al., 1997). Crooked calf disease is a condition in which calves are born with a variety of deformities such as arthrogryposis, scoliosis, kyphosis, torticollis, and cleft palate (Shupe et al., 1967a,b, 1968). On lupine-infested rangelands, the incidence of crooked calf disease is often 1 to 5% and can exceed 30% of pregnant animals (Panter et al., 1997; Gay et al., 2004, 2007).

Ingestion of toxic plants may alter diet selectivity and forage intake of herbivores (Provenza et al., 1992; Forbes and Kyriazakis, 1995), and in turn, the ability of herbivores to detoxify poisonous plants may be influenced by their nutritional or physiological status (Boyd and Campbell, 1983; Foley et al., 1995). Well-nourished animals may effectively process toxins from plants, whereas nutrient-deprived animals may be more likely to eat poisonous plants (Lopez-Ortiz et al., 2007) or they may be metabolically compromised in their ability to detoxify or excrete the toxins (Foley et al., 1995; Lopez-Ortiz et al., 2004).

[1]The authors thank Nicole Dye, Terrie Wierenga, and Kermit Price (USDA-ARS Poisonous Plant Research Laboratory, Logan, UT) for technical assistance.

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Received September 26, 2007.
Accepted May 30, 2008.

The objective of this experiment was to determine whether body condition would affect serum alkaloid concentration of the teratogenic quinolizidine alkaloid (anagyrine) in velvet lupine (*Lupinus leucophyllus*) or the teratogenic piperidine alkaloid (ammodendrine) in sulfur lupine (*Lupinus sulphureus*) in cattle.

MATERIALS AND METHODS

All procedures involving animals were approved by the Utah State University Institutional Animal Care and Use Committee.

Plant Collection

Velvet lupine (*L. leucophyllus*) was collected 16 km southeast of Ritzville, WA (W118° 15′ 00″; N46° 56′ 50″). The plant was collected in mid June, 2002 during full flower/early pod phenological growth stage. Sulfur lupine (*L. sulphureus*) was collected 14.5 km northeast of Ritzville, WA, on the Lincoln and Adams county line (W118° 15′ 00″; N47° 15′ 50″). The plant was collected at the end of May, 2002 while in late seed pod/early pod shatter stage of phenology. Both plant collections consisted of all above-ground plant parts (aerial). Plant material was dried at ambient temperature, then ground through a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ). The ground plant was stored in plastic bags at ambient temperature until used.

Animal Conditioning Period

Eight mature (3 to 6 yr old) lactating Hereford cows were randomly assigned to 1 of 2 groups to attain low (**LBC**; n = 4) or high body condition (**HBC**; n = 4). Calves from cows assigned to the HBC group were weaned immediately before conditioning to remove the energy demand of lactation and promote BW gain and improve body condition. Calves remained with cows in the LBC group until the end of the conditioning period (60 d) to maintain the energy demands of lactation, and then calves were removed 6 mo before cows were dosed.

During conditioning, all cows were housed individually in a barn with free access to water and mineralized salt. Cows assigned to attain LBC were fed a daily ration of a low nutrient diet equivalent to 1.9% of their BW (DMI). This diet consisted (as-fed basis) of a mixture of hay (10% alfalfa, 25% grass), wheat straw (60%), soybean meal (5% soybean), and contained 8.3% CP and 52% TDN. Cows assigned to attain HBC were offered ad libitum access to a high nutrient diet. This mixed diet (as-fed basis) consisted of alfalfa hay (60%), soybean meal (10%), rolled corn (25%), and molasses (5%), resulting in a diet with 19.7% CP and 72% TDN. An experienced observer, who was blind to the treatments, scored the cattle individually every 2 wk by visual estimation of fat deposition on a 9-point scale (1 = thin; 9 = obese; Momont and Pruitt, 2002). After conditioning, cows were grazed together on cheatgrass-dominated rangelands in eastern Washington for 1 mo, then group-fed alfalfa hay at 1.5% BW (as fed basis) until the end of the study. At the time of dosing *L. leucophyllus*, the LBC animals had average BW of 406 ± 13 kg and a BCS of 2.8 ± 0.3. The HBC group had an average BW of 645 ± 95 kg and a BCS of 7.9 ± 0.3. After 21 d, and at the time of dosing *L. sulphureus*, the average BW of the LBC group was 425 ± 18 kg and the BCS was 2.6 ± 0.6. For the HBC group, at dosing the average BW was 663 ± 98 kg and the BCS was 7.6 ± 0.3.

Experimental Procedure

Plant material was dosed at a level previously observed to cause mild overt clinical signs, including muscular weakness, salivation, and relaxation of the nictitating membrane of the eye (Panter et al., 1992). Each cow was given a single dose (2 g/kg of BW) of ground *L. leucophyllus* at 0800 h. The ground lupine was diluted in 10 L of warm tap water and gavaged directly into the rumen using a livestock pump system (The Magrath Company, McCook, NE). After 21 d, cows were reweighed and BCS determined before dosing with the second lupine species (*L. sulphureus*) with the same dose and methods as outlined above. After oral gavage, blood samples (30 mL) were drawn via jugular venipuncture at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 54, and 60 h. Blood was allowed to clot for 1 h at room temperature, centrifuged for 30 min at 1,060 × *g,* and serum was immediately harvested and frozen in an ultra-cold freezer (–50 to –60°C) for future alkaloid analysis.

Plant Alkaloid Analysis

Extraction of plant material (*L. leucophyllus* and *L. sulphureus*) for alkaloid analysis was accomplished by weighing 100 mg of ground lupine plant material into a 10-mL glass screw-top test tube equipped with teflon-lined caps. Five milliliters of 1 *N* HCl and 4 mL of $CHCl_3$ were added to the test tubes and placed in a mechanical shaker for 15 min, then centrifuged to separate the aqueous and organic phases. The aqueous fraction was transferred to a clean glass test tube and basified to a pH between 9 and 9.5 by drop-wise addition of concentrated NH_4OH. The basified solution was extracted twice with $CHCl_3$ (4 mL, then 2 mL). The combined $CHCl_3$ fractions were filtered through anhydrous Na_2SO_4 and then dried under a stream of N_2 at 50°C to yield the crude alkaloid fraction.

The alkaloid fraction extracted from *L. leucophyllus* was reconstituted in 4 mL of methanol containing 1.3 μg/mL of caffeine (internal standard). A portion (~1 mL) was transferred to 1.5-mL gas chromatograph auto sample vial and 2 μL was injected into an HP 5890 gas chromatograph (HP 5890, Agilent, Palo Alto,

CA) equipped with a split/splitless injector, FID detector, and a J&W DB-5 (30 m × 0.33 mm i.d.) capillary column. The carrier gas was helium at a flow rate of 1.5 mL/min. Injector temperature was 250°C and operated in the splitless mode. Split vent flow rate was 60 mL/min and purged after 1 min. Oven temperature was programmed: 100°C for 1 min, 100 to 200°C at 50°C/min, and 200 to 320°C at 5°C/min. Caffeine was used as an internal standard and plant alkaloid peaks were quantified against a 6-point anagyrine standard curve over the range of 800 to 25 μg/mL of anagyrine in methanol prepared by serial dilution.

The alkaloid fraction from *Lupinus sulphureus* was reconstituted in 4 mL of methanol. A 1-mL aliquot was diluted 50% by the addition of 1-mL methanol and then a 30-μL aliquot was added to 90 μL of a 20 m*M* ammonium acetate solution containing 1.3 μg/mL of caffeine (internal standard) in a 150-μL autosample vial insert. Quantitative analysis of the alkaloids was made using reversed phase HPLC-MS/MS. A 50-μL aliquot was injected onto a Betasil C8 reversed phase column (100 × 2.1 mm i.d.; Thermo Electron Corporation, Waltham, MA) protected by a guard column of the same adsorbent. The alkaloids were eluted from the column with a gradient flow (0.250 mL/min.) of 20 m*M* ammonium acetate in water (solvent A) and methanol (solvent B) delivered by a Hewlett Packard 1100 series binary HPLC pump. The mobile phase program was started at a composition of 95:5 (A:B) and then changed by linear gradient to 85:15 over 15 min, followed by a second linear gradient to 30:70 (A:B) during 15 to 30 min of the run. The HPLC system was re-equilibrated for 15 min at the initial mobile phase composition of 95:5 (A:B) before the next injection. The total HPLC run time was 45 min. Detection was performed using a Thermo Finnigan (San Jose, CA) LCQ ion trap mass spectrometer. Ionization was achieved using an atmospheric pressure chemical ionization source with a vaporizer temperature of 450°C and corona discharge current of 5 μamps. The capillary inlet temperature and voltage were 200°C and 15 V. Operational parameters of the mass spectrometer were optimized using the standard "auto tune" procedure using solutions of anagyrine and ammodendrine added to the LC solvent flow 75:25 (A:B) via syringe pump to obtain maximum ion current of the respective MH^+ ions for the alkaloid of interest. The mass spectrometer was run in an MS/MS mode, scanning product ions from a mass range of 70 to 300 amu after fragmentation of the caffeine, ammodendrine and anagyrine protonated molecular ions [MH^+ = 195 (caffeine), 209 (ammodendrine), 245 (anagyrine)] using a relative collision energy setting of 35% (caffeine, anagyrine) and 25% (ammodendrine). Maximum ion trap inject time was 500 ms, and 2 microscans were averaged for each data point. Caffeine was used as an internal standard and plant alkaloid (ammodendrine) concentration was quantified against a 5-point standard curve over the range of 53.2 to 3.33 μg/mL of ammodendrine in methanol.

Serum Alkaloid Analysis

The sera were thawed, thoroughly mixed by vortexing, and then centrifuged at 1,360 × *g* for 30 min. A 5-mL aliquot of sera was transferred to a clean 10-mL glass test tube. Fifty microliters of 85% phosphoric acid was added to each sera sample and mixed well. Using a Supelco Visiprep 24 vacuum manifold (Supelco, Bellefonte, PA), the serum samples were each applied to a Strata X-C; 60 mg/3 mL SPE cartridge (Phenomenex, Torrance, CA) that had been conditioned with 2 mL of MeOH followed by 2 mL of distilled deionized H_2O. Each cartridge was washed with 2 mL of 0.1% H_3PO_4, dried under 10 mmHg for 3 min, and washed with 2 mL of MeOH. The cartridge was eluted twice with 1.5 mL of 5% NH_4OH/MeOH solution (3 mL of total volume) to remove the alkaloids from the SPE cartridges. The NH_4OH/MeOH solution was evaporated to dryness under a gentle flow of nitrogen in a heating block at 60°C. The residue from each sample was dissolved in 15 μL of methanol to yield an analytical stock sample, which was kept at 4°C. Before analysis, samples were prepared by adding a 30-μL aliquot of the analytical stock sample to 90 μL of a 20 m*M* ammonium acetate solution containing 1.3 μg/mL of caffeine in a 150-μL autosample vial insert. A 50-μL aliquot was injected into the HPLC-MS/MS system and concentrations of anagyrine and ammodendrine were determined by reversed phase HPLC-MS/MS method described previously. Anagyrine in sera samples were quantified against a 7-point standard curve over the range of 0.800 to 0.0125 μg/mL of anagyrine in sera. Ammodendrine in sera samples were quantified against an 8-point standard curve over the range of 1.600 to 0.0125 μg/mL of ammodendrine in sera.

Toxicokinetic Evaluation

Absorption and elimination profiles were analyzed using standard pharmacokinetic software (PK Solutions for Non compartmental Pharmacokinetic Data Analysis, Summit Research Services, Montrose, CO; 1998). A curve-stripping procedure was used to determine the basic pharmacokinetic variables of half-life, rate, and blood concentration intercept for each phase of the blood level curve. The following variables were determined for ammodendrine and anagyrine:

Intercept: C_n = coefficient of each exponential term;

Slope: $s = -\lambda_n/2.303$;

Rate constant: $\lambda_n = -2.303s$;

Half-life: $t1/2 = 0.693/\lambda_n$;

C_{max} = maximum observed concentration;

T_{max} = time point at C_{max}; and

Area under the curve (**AUC**) ∞ (area) A Trapezoidal method was used to determine the AUC of a concentration vs. time graph.

The general equation describing the disposition of xenobiotics in serum is given by the summation expression: $C = \Sigma C_n \exp(-\lambda_n t)$ where Cn and λn are the zero-time intercepts and rate constants, respectively, for each exponential term. The corresponding triexponential function can be written as $C = Ae^{-\alpha t} + De^{-\beta t} + Ee^{-\gamma t}$. In this expression, the intercepts are given letter designations indicating the 3 common drug disposition phases of absorption (**A**), distribution (**D**), and elimination (**E**) that are typically encountered after an oral dose.

Statistical Analyses

Each lupine species was considered a separate study. Each experiment was a completely random design, with repeated measures over time on each animal. The model included treatments, animals nested within treatments, time, and all interactions. Animals were considered a random factor. Mixed-model analyses of variance for correlated repeated measures within subjects (PROC MIXED, SAS Inst. Inc., Cary, NC) were performed to determine the effect of body condition on serum alkaloid concentrations. Variances were tested for homogeneity, and various options were used to model the covariance structure. Typically the compound symmetry option provided the best fit.

Area under the curve and other kinetic variables for ammodendrine and anagyrine in serum were analyzed separately. Because these variables did not have a time component, they were analyzed using *t*-tests.

RESULTS

The *L. leucophyllus* dosed in this study contained 0.27% anagyrine (DM basis), whereas the *L. sulphureus* contained 0.42% ammodendrine (DM basis). Cows were dosed by mass of plant material (2 g/kg of BW) or the equivalent of 5.4 mg of anagyrine/kg of BW in the first experiment and 8.4 mg of ammodendrine/kg of BW in the second experiment. Body condition influenced the serum concentration of both teratogenic alkaloids, anagyrine and ammodendrine, from *L. leucophyllus* and *L. sulphureus,* respectively.

Anagyrine

There was a time × treatment effect on anagyrine concentration ($P = 0.001$). High body condition cows had a mean serum anagyrine concentration of 0.20 μg/mL compared with 0.07 μg/mL for LBC cows, and differences varied over time (Figure 1). High body condition cows differed ($P < 0.04$) from LBC cows at h 1, 2, 3, 4, 6, 8, and 12. From h 18 ($P = 0.07$) and onward, no differences were detected.

The toxicokinetic profiles of anagyrine were different between HBC and LBC cows (Figure 2). The overall kinetic profile of serum anagyrine, as described by the AUC (a kinetic parameter that is a measure of the total amount of xenobiotic that reaches the systemic circulation) tended to differ ($P = 0.11$) between HBC cows (AUC = 8,258 ± 4,533) vs. LBC cows (AUC = 3,410 ± 509). There were differences ($P = 0.02$) in the maximum serum anagyrine concentration (C_{max} = 538 ± 159 for HBC cows vs. C_{max} = 182 ± 23 for LBC cows). Absorption of anagyrine tended ($P = 0.11$) to be faster in HBC cows (A1/2 = 1.7 ± 1.2 for HBC cows vs. A1/2 = 2.7 ± 0.7 for LBC cows). Similarly, there was a trend ($P = 0.15$) toward differences in elimination half life between the HBC and LBC groups (E1/2 = 7.8 ± 0.8 for HBC cows vs. E1/2 = 9.6 ± 2.0 for LBC cows) However, there was no difference ($P > 0.45$) in distribution of anagyrine (D1/2 = 3.4 ± 1.7 for HBC cows vs. D1/2 = 3.9 ± 1.4 for LBC cows).

Ammodendrine

There was a time × treatment interaction ($P = 0.001$) on ammodendrine concentration. High body condition cows had a mean serum ammodendrine concentration of 0.59 μg/mL compared with 0.30 μg/mL for LBC cows, and differences varied over time (Figure 1). Ammodendrine concentration in serum of HBC cows differed ($P < 0.03$) from LBC cows at h 2, 3, 4, 6, 8, and 12. From h 18 ($P = 0.08$) and onward, no differences were detected.

The kinetic profiles of ammodendrine are shown in Figure 3. Area under the curve for the ammodendrine kinetic profile tended to be greater ($P = 0.1$) in HBC cows (AUC = 21,048 ± 10,109) than for LBC cows (AUC = 9,980 ± 2,187). There were also differences ($P = 0.06$) in the maximum serum ammodendrine concentrations (C_{max} = 1,631 ± 509 for HBC cows vs. C_{max} = 924 ± 218 for LBC cows). Even though C_{max} and AUC values for ammodendrine tended to be greater for HBC cows, the distribution half life (D1/2 = 3.8 ± 2.3, HBC vs. D1/2 LBC = 1.6 ± 0.3, LBC) was greater ($P = 0.02$) in the LBC cows. The elimination half-life (E1/2) tended to differ ($P = 0.12$) between the HBC (4.1 ± 1.2) and LBC (2.9 ± 0.1) groups, whereas there was no difference ($P = 0.32$) in the absorption half life (A1/2) between HBC (2.3 ± 0.07) and LBC (1.8 ± 0.3) groups for ammodendrine.

DISCUSSION

This study showed that body condition of cows affected serum alkaloid concentration and tended to affect absorption and elimination of 2 teratogenic alkaloids (anagyrine and ammodendrine). The quinolizidine alkaloid, anagyrine (Keeler, 1976), and some piperidine alkaloids including ammodendrine (Keeler and Panter, 1989; Panter et al., 1998) have been shown to reduce fetal movement during gestation (Panter et al., 1990; Panter and Keeler, 1992). Therefore any changes to an

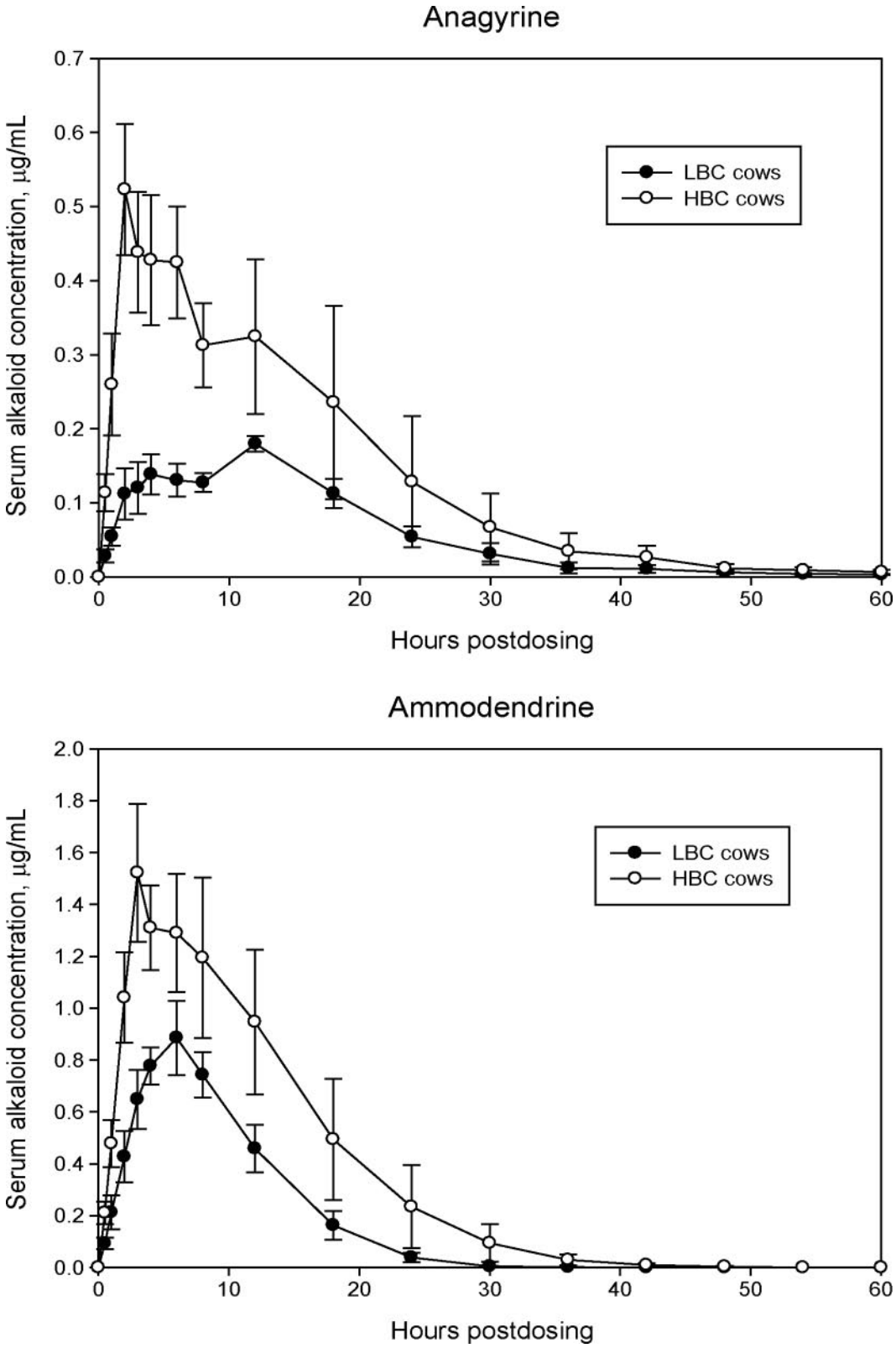

Figure 1. Concentration of anagyrine and ammodendrine in blood (mean ± SE) from low body condition (LBC) and high body condition (HBC) cows dosed with 2 g of ground *Lupinus leucophyllus* (anagyrine) and *Lupinus sulphureus* (ammodendrine)/kg of BW.

animal's physiological status, such as a change in body condition, could alter the disposition of teratogenic alkaloids potentially impacting the incidence of crooked calf disease. Although some work has been conducted in sheep (Lopez-Ortiz et al. 2004), this is the first study to report the relationship between body condition and

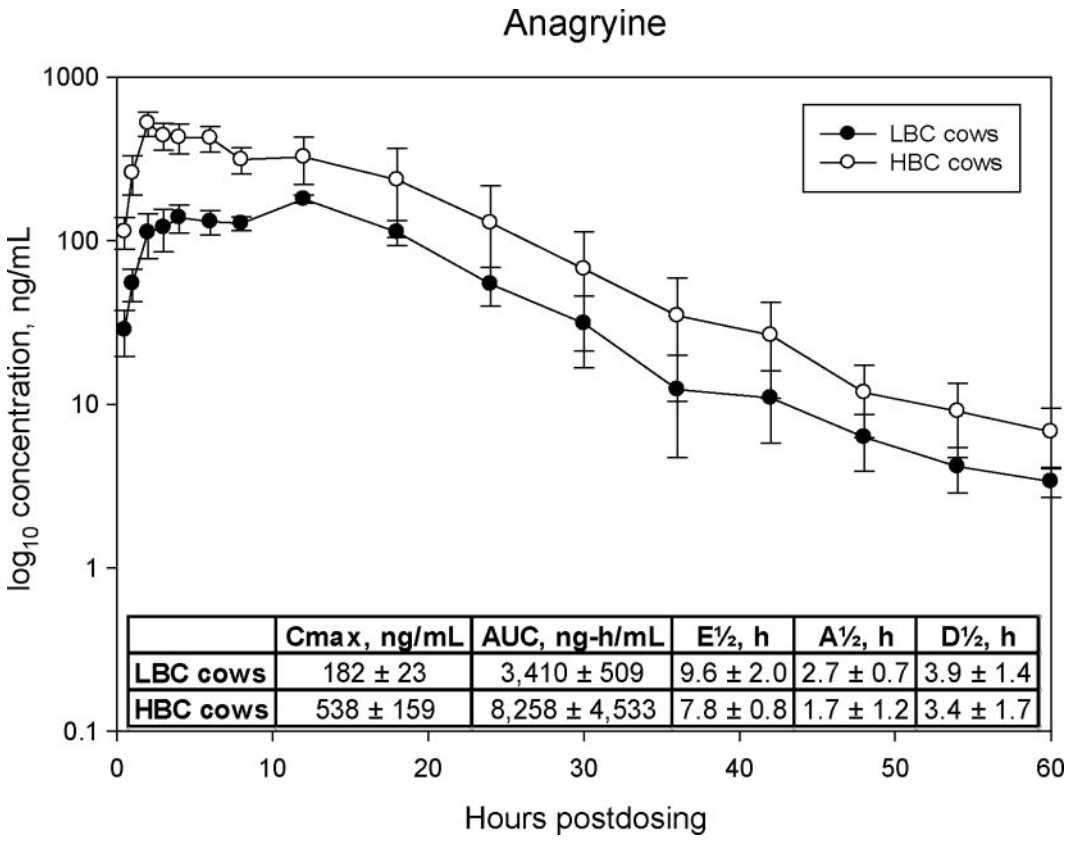

Figure 2. Anagyrine kinetic profile of low body condition (LBC) and high body condition (HBC) cows dosed with 2 g of ground *Lupinus leucophyllus*/kg of BW. The data represent the serum anagyrine concentrations plotted as a semi-logarithmic graph. C_{max} (maximum observed concentration), AUC (area under the curve), E1/2 (elimination half life), A1/2 (absorption half life), and D1/2 (distribution half life) were calculated using the software PK Solutions 2.0 (Summit Research Services, Montrose, CO). Data are presented as mean ± SE.

serum alkaloid concentration of these lupine toxins in beef cows.

An overall comparison of all kinetic variables indicate that the HBC cows tended to absorb more of the teratogenic alkaloid anagyrine than LBC cows, and they did so faster. Area under the curve tended to differ ($P \leq 0.11$) between HBC and LBC cows for both alkaloids. The HBC group had 2.4 times as much bioavailable anagyrine as indicated by the AUC as the LBC group. Additionally, the maximum blood anagyrine concentrations (C_{max}) were 3 times as high in the HBC group. Interestingly, there was no difference in the rate of elimination of anagyrine between the HBC and LBC groups.

Cattle grazing lupine-infested rangelands show tremendous variation in observable signs of intoxication and incidence of crooked calf disease, perhaps due in part to physiological variation in response to lupine alkaloids (Lopez-Ortiz et al., 2007). This variation was also evident in this study. For example, for anagyrine the T_{max} ranged from 2 to 12 h in the HBC group and 3 to 12 h in LBC group, C_{max} ranged from 168 to 223 ng/mL in LBC cows and 277 to 700 ng/mL for the HBC cows, and the AUC ranged from 2,975 to 4,249 in the LBC group and 3,378 to 15,353 in the HBC group. The small sample size undoubtedly affected statistical significance, with several toxicokinetic parameters tending toward significance. One cow in each group differed markedly from the other animals in their respective treatment group. Although these 2 cows were in different body condition (BCS 7.5 vs. 2.5), they had similar values for C_{max} and AUC, indicating that if they had been pregnant their fetuses may have been exposed to nearly equal amounts of anagyrine.

In general, body condition had a similar effect on the kinetic profiles for ammodendrine as seen with anagyrine. The AUC and C_{max} for ammodendrine were 2.1-fold and 1.8-fold greater in HBC than LBC cows, respectively. One interesting and potentially important difference was that the rate of elimination for ammodendrine tended to be greater for the LBC cows. The difference in the rate of elimination for ammodendrine in the LBC cows may be important because it indicates that ammodendrine may be cleared from the LBC cows sooner than the HBC cows.

The C_{max} and AUC for anagyrine and ammodendrine were greater in the HBC than LBC cows. This could be due in part to the fact that the alkaloids were administered according to BW, and consequently HBC cows received a greater amount of alkaloids. The difference in

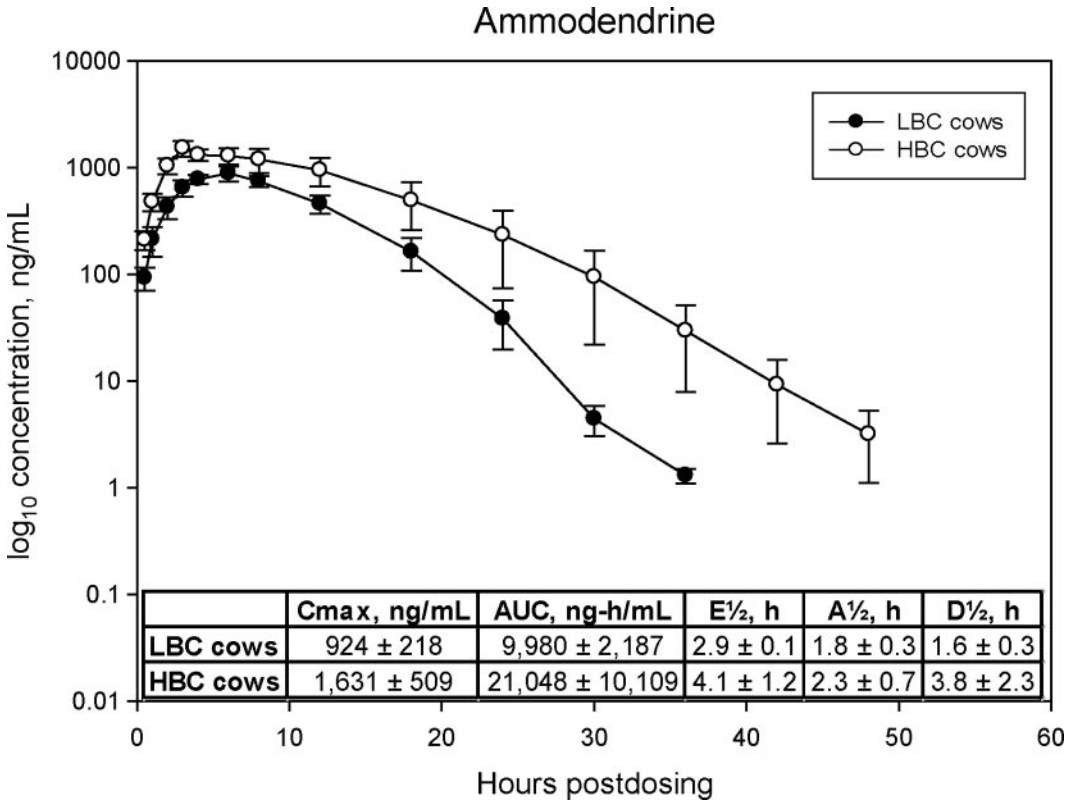

Figure 3. Ammodendrine kinetic profile of low body condition (LBC) and high body condition (HBC) cows dosed with 2 g of ground *Lupinus sulphureus*/kg of BW. The data represent the serum ammodendrine concentrations plotted as a semi-logarithmic graph. C_{max} (maximum observed concentration), AUC (area under the curve), E1/2 (elimination half life), A1/2 (absorption half life), and D1/2 (distribution half life) were calculated using the software PK Solutions 2.0 (Summit Research Services, Montrose, CO). Data are presented as mean ± SE.

dose received does not, however, account completely for differences in the kinetic profiles. The HBC group was dosed with 1.6 times the alkaloids as the LBC group, however, there was over a 2-fold difference in the AUC for both alkaloids between the 2 groups.

In previous work with sheep dosed with silvery lupine (*Lupinus argenteus*), AUC, and peak concentrations of anagyrine were greater for LBC sheep compared with moderate-condition animals (Lopez-Ortiz et al., 2004). Further, serum elimination of anagyrine was decreased in LBC vs. moderate body condition sheep. These results for LBC sheep and anagyrine are in direct contrast to the present cattle study. Low body conditioned sheep may simply metabolize and excrete anagyrine differently than do LBC cattle. This is supported by the fact that anagyrine is teratogenic in cattle but not in sheep (Keeler, 1988). Whether this difference is metabolic or results from other factors is not known at this time.

Previous studies have shown that body condition may be important in not only inducing cattle to graze certain plants but also influences the length of time they graze these plants. Launchbaugh et al. (2007) demonstrated that body condition, nutritional status, or both had a major impact on not only amount of plant consumed but also on amount of time spent grazing. Previous work with cattle in HBC and LBC has shown that when HBC and LBC cows are exposed to lupine-infested pastures, LBC cows began to eat lupine sooner after turnout (d 1 vs. 4), and LBC cows also had a higher rate of lupine ingestion compared with HBC cows (Lopez-Ortiz et al., 2007). This is especially important with lupine-induced crooked calf disease because cows must graze the plant over a prolonged period of time (1 to 3 wk to inhibit fetal movement over the critical gestational period) without interruption for the calf to manifest the contracture defects. Even though HBC cows tend to have greater absorption and slower elimination rates of toxic alkaloids than do LBC cows, under grazing conditions they may eat less lupine than do LBC cows (Lopez-Ortiz et al., 2007); thus, susceptibility depends on the final balance between ingestion and kinetics of lupine alkaloids. Clearly, more work is required to determine susceptibility as related to body condition.

Results of this research demonstrate the importance of body condition on absorption, distribution, and excretion of teratogenic lupine alkaloids. Serum alkaloid concentrations of the 2 teratogenic alkaloids from *L. leucophyllus* and *L. sulphureus* were clearly affected by cow body condition. This is important for grazing animals and the extent to which they are affected by plant

toxins. Even more significant is the consideration of fetal insult to cows grazing teratogenic lupines during d 40 to 100 of gestation where sustained suppression of fetal movement is the primary factor in the induction of crooked calf disease (Panter et al., 1990; Panter and Keeler, 1992).

Lupine-induced crooked calf disease remains an important obstacle to optimum range utilization in the western United States. Body condition may have a profound influence on toxic effects from livestock ingesting some plants. Whereas lupines containing teratogenic alkaloids have been responsible for substantial calf losses, the nutritional value of lupine late in the grazing season cannot be ignored. Therefore, management programs must be adjusted to enhance utilization of these ranges and exploit the benefits of lupines but reduce the losses. We recommend that pregnant cows be maintained in at least moderate body condition, with the understanding that these cows are still very susceptible to lupine-induced teratogenesis. Even with increased understanding of the kinetics of these 2 teratogenic alkaloids in relation to body condition, ranchers should utilize all available options to avoid grazing pregnant cows on lupine ranges from gestation d 40 to 100 to avoid crooked calves.

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